# EFFECT OF EXTERNAL STIMULI ON THE DIELECTRIC PROPERTIES OF MICROTUBULES IN THE GIGAHERTZ RANGE

A Synopsis submitted in the partial fulfilment of the requirement for the award of the degree of

**Doctor of Philosophy**
**in**
**Physics and Computer Science**

Submitted by
**Komal Saxena**

Under the Supervision of
**Dr. K. Soami Daya**

**Prof. Ravindra Kumar**
Dean,
Faculty of Science

**Prof. G.S. Tyagi**
Head,
Dept. of Physics & Computer Sc.
Faculty of Science

DEPARTMENT OF PHYSICS AND COMPUTER SCIENCE
FACULTY OF SCIENCE
DAYALBAGH EDUCATIONAL INSTITUTE
DAYALBAGH
AGRA (UP) – 282005

MARCH 2015

## 1. RATIONALE

Microtubule (MT) is one of the basic subcelluar structures of cytoskeleton, present in all eukaryotic cells. It is formed by highly electrically polar α and β tubulin hetrodimer [1 – 8] which generate varying electric fields [9] [10] [11]. MT plays an important role in information processing in the cell [11] and also governs motion [4], morphogenesis [13 – 18] and reproduction [13] [14] [18] [19]. Unique signature of aggregation and dispersion of tubulin molecules from nm to hundred of μm make them particularly interesting for carrying out the dielectric studies. MTs have been associated with higher level cognition [11] and are observed to be resonant in the microwave region. These high frequency resonance foot prints of MT motivated me to explore the dielectric properties of microtubules in the gigahertz frequency range.

## 2. MICROTUBULE IN EUKARYOTES

MT is a vital subcelluar biomolecule found in almost all living systems. It is formed by a folded 2D sheet of tubulin hetrodimer subunits α and β, and creates hollow cylindrical shape with the association of 13 parallel protofilament [1]. These hetrodimers are highly polarized in nature [1 – 7]. Each tubulin have 55000 Dalton molecular weight and a diameter of 4 – 5nm.These dimers polymerize to form MT. End to end polymerization is present in MT, where one end is exposed by α subunit and other is exposed by β subunit with opposite polarity. Negative end of MT is bound by centrosome and positive end is free toward the cytoskeleton. [1] Outer layer and inner layer of microtubule are 25 nm and 15 nm in diameter respectively. [1] [7] Length of MT is instable. By introducing the synchrony and de-synchrony with the combination of a catalyst, growth process and dynamic behaviour of MT can be effected. MT grows to its longer limit in case of stabilizer (positive catalyst) and shows the reverse response in case of de-stabilizer (negative catalyst). Limit length of microtubule are independent of the density of tubulin protein. [7] MT in eukaryotic cells is generally realized by three-start helix [1] [7]. Based on their interaction between subunits, two types of systems are presented. In one

type of system αβ subunit is adjacent to βα subunit while in second system αα subunit is adjacent to ββ subunit [8]. Dynamic stability is present in MT structure [4].

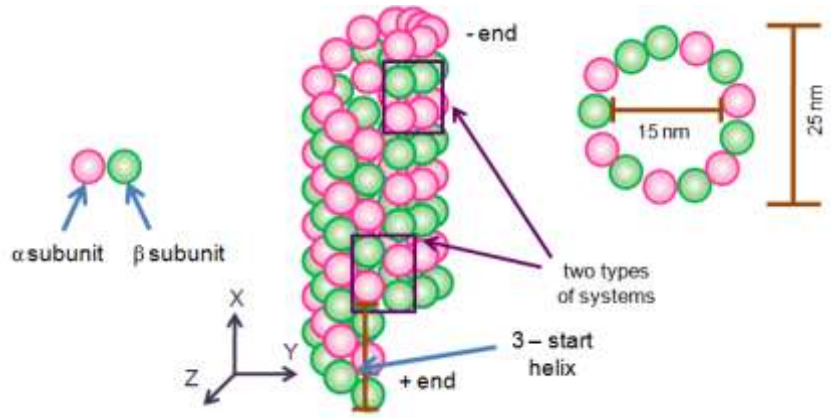


Fig. 1 2D folded sheet of α β tubulin dimers constructed microtubule from 13 protofilaments in eukaryotic cells. Inner and outer diameters of microtubule are 15 nm and 25 nm respectively. 3 – Start helix and two types of systems having different adjacent property are also represented

## 2.1 OSCILLATING ELECTRIC FIELD OF POLARIZED MT

MTs are composed by electrically polar tubulin sub-units. Dipole moment is calculated by various researchers. Dipole moment of each subunit is calculated by Mershin et al. [2]. Detailed description of dipole moment is given by Tuszynski et al. [3]. They calculate the dipole moment along the axis. Along X, Y and Z axis, value of dipole moments are 337 D, -1669 D, and 198 D respectively. The dipole moment may arise due to the motion of electrons and generate electric field. By taking the vector sum of all fields due to dipoles, Total field is analyzed [9]. The generated electric field can be analyzed by many groups [4 – 10].

Many sources are present which provides energy to microtubule [9] [10] [11]. Waste energy of mitochondria excites the vibration of MT into highly non-linear region [11]. The internal process of MT as dynamic instability also provides the energy at the time of hydrolysis of GTP [9] [10]. Another cause of excitation of vibration in MT is movement of motor protein and interaction with MT. Motor proteins contribute to transfer the energy [9] [10]. Therefore, mechanical vibrations of the microtubule are correlated with electrodynamic field.

Depending upon the dipole direction, multiple conducting states are formed in MT. Therefore, MT can behave as a memory-switching element [21].

### 2.1.1 EFFECTS OF EXTERNAL STIMULI

Effect of external stimuli like electric field, light, drugs and temperature has been extensively studied on microtubules. An extensive review of external stimuli on MTs is discussed in this section.

**A) Effect of electric fields on MT**

When external field is applied on MT, the vibration of MT is changed and affects other vital properties of MT. These properties are studied by several researchers [7] [8] [13 – 16]. Therefore in Table 1, list of the researchers who seen the effect of electric field on MT, which properties they have measured, which method is used and at which frequency they have done their experiment are given.

**Table 1 : List of research work reported on the effect of electric field as stimuli on MT**

| Researchers | Property measured | Method | Frequency |
|---|---|---|---|
| Vassilev et al. (1982) [12] | • Alignment of parallel arrays of MTs | Application of electric field on the order of 25nm/cm | 10 Hz |
| Kirson et al. (2004) [13] | • Mitosis effect in cancer cells | AC electric field ~ 2 V/cm | 100 – 300 KHz |
| Minoura and Muto (2006) [14] | • Ionic conductivity – 150 mS/m at 1MHz,<br>• resistance – 27 GΩ/μm<br>• Induced dipole moment arising | Application of electric field on the order of ($0.5–1.9 \times 10^5$ V/m) by Electro-orientation method | 10 KHz – 3 MHz |
| Samsonov, A. | • Assembly or | Application of EM field, | 94 GHz |

| | | | |
|---|---|---|---|
| and S. V. Popov (2013) [15] | disassembly of MTs in "Xenopus spinal cord". | Two glass coverslips, separated by a thin layer of cell culture medium, were mounted on the inverted microscope. Waveguide is placed above. | |
| Sahu et al. (2014) [7] | • Self-assembly of protofilament and found that at 37 MHz it speed up.<br>• Rapid mode and silent mode | Triggered ac signal by using nano-electronic set up | Hz – GHz |
| S. S. Setayandeh and A. Lohrasebi (2015) [8] | • Young modulus and flexibility. Lower at 2, 5, or 7 GHz system become more rigid.<br>• At 1 or 6 GHz system become more flexible. | 0.03 V/mm (Computational based) | 1 – 10 GHz |

**B) Effect of drugs on MTs**

Neuro-degenerative diseases and cancer are associated with the dynamic behaviours of MTs. Therefore, in chemotherapy and other treatments, different drugs can be used to regulate the MT instability. Many researchers have worked on it [7] [17] [18]. Table 2 summarize the list of not all but few researchers who are measuring the effects of drugs on MT.

**Table 2 : List of research work reported on the effect of drug as stimuli on MTs**

| Researchers | Properties Measured | Method |
|---|---|---|
| Dye et al. (1993) [17] | • Flexibility and limping is shown | Taxol effect on MT |
| P. Kovács & G. Csaba (2005) [18] | • Change in MTs assembly | Use different drugs –<br>Colchicines (reduce polymerization, binding process is slow)<br>Vinblastine (de-polymerize MT)<br>Nacodazole (binds β tubulin)<br>taxol (stabling MT) |
| Sahu et al (2014) [7] | • Regulate MT dynamics, growth rate and length limit | Add positive (acts as stabilizer) and negative catalyst (acts as de-stabilizer) to synchrony detection circuit |

Based on this, S. S. Setayandeh and A. Lohrasebi recently proposed in their paper that taxol in chemotherapy can be replaced by electric field at GHz frequency [8].

**C) Effect of light on MT**

Exposure of microtubules to light also influences its properties. Fragmentation and integration process in MT are happened in the presence of UV light. And when white light is introduced, MTs of guard cell are arranged parallel. In dark it shows random behaviour. Responses of MTs are different at different wavelength [18] [19] [21] [22].

**Table 3 : List of research work reported on the effect of light as stimuli on MTs**

| Researchers | Properties Measured | Wavelength Used |
|---|---|---|
| Zaremba et al. (1984) [18] | • Damage of microtubulin protein (calf brain).<br>• Small amount of mitotic delay | UV radiation (mercury-xenon lamp), |
| R., Veselská and Janisch R. (2000) [19] | • MTs is sensitive than microfilaments.<br>• fragmentation process - Reduced the no. of microtubules (short periods)<br>• restore MT | UV radiation ( balzers lamp at 356 nm) |
| Lahav et al. (2004) [21] | • In the presence of light, MTs (guard cell) are arranged in parallel.<br>• In dark, MTs are randomly oriented. | White light |
| | • Resembled dark effect | red light |
| | • Resembled white effect | Blue light |
| Sambade et al. (2012) [22] | • Modify the dynamics of microtubule by light –signalling pathway<br>• Ability to switch between orthogonal axes. | 488 nm diode laser |

**D) Effects of sound on MTs**

Todate no literature has been found on the effect of sound signal on different electrical or mechanical properties of MTs. However, the recent work of Sahu et al. shows resonance peaks in tubulin and microtubules in the audible frequency range [24]. Therefore, in this study we propose to analyse the effect of weak audio signals on dielectric properties of microtubules.

## 3. MICROWAVE TECHNIQUES FOR DIELECTRIC CHARACTERIZATION

Accurate knowledge of the complex relative permittivity of biological tissues is necessary to evaluate biological effects as well as the efficiency of medical applications. The complex

permittivity – dielectric constant and losses – of a material is almost always measured by inserting a specimen into a capacitor, waveguide, or cavity which forms part of an electrical circuit. For frequencies up to 1GHz resonant circuits may be used, the specimen being combined with a known inductance in the form of a Q-meter using essentially a series combination of C, L, and R. The energy absorption exhibits a resonant peak and the width of this peak $2\Delta\omega$ is measured, where $\Delta\omega$ is the difference between the frequency where the energy loss is a maximum and the frequency where it is reduced to half the maximum. . Leads can be avoided, for instance, by using a capacitance whose value can be modified by micrometer screws. This, however, makes the method difficult to use over more than a narrow range of temperatures. [25]

Scattering matrix are most often used for calculating and estimating the errors. They describe a two-port by its reflection and transmission characteristics. When measuring the electric properties of a dielectric, the container in which the specimen is placed is considered as the electrical two- port.

For the present study we propose to carry out the studies using cavity perturbation method with a dielectric resonator. In the proposed work, a novel approach for the investigation of aqueous solutions interacting with electromagnetic radiation at micro-to-millimeter wave frequencies is proposed with relevance to biological applications, on the basis of simulated results using CST Microwave Studio.

## 4. OBJECTIVE

Proposed doctoral work aims to study the dielectric properties of microtubules in the presence of external stimuli. The specific objectives are:

I. Dielectric characterization of microtubules through resonant perturbation method at different frequencies in the GHZ range.

II. Understanding the effect of following external stimuli on the dielectric properties of MTs through secondary perturbation:

   i. Weak AC signals Varying from 3 Hz to 30 GHz.
   ii. Effect of monochromatic light signal from IR to UV region.
   iii. Effect of analog sound signals sampled at different rates.
   iv. Broadband reflection imaging of MTs in the presence of external stimuli.

# 5. METHODOLOGY

Methodology for the proposed doctoral work is divided into following steps:

**A) Simulation:**

Study of dielectric properties of microtubules will be carried out by simulating virtual experimental design on the Microwave Studio Suite. Different designs will be simulated and design optimization will be carried out.

**B) Design**:

From the optimization study carried out in step-A the best design will be fabricated.

**C) Experimentation:**

This step will be carried out in following sub-steps

   i. Dielectric characterization of pure MTs in using primary perturbation
   ii. Effect of external stimuli using secondary perturbation
   iii. Design and experimentation on broadband reflection studies for dielectric imaging

## 6. EXPECTED OUTCOME

Present research study will attempt to study the dynamic instability of microtubules due to aggregation and dispersion of polar α and β tubulin molecules which results in rapid and out of equilibrium changes in the polarization. Further the study will experimentally observe the effect of weak signals of light, sound and AC fields on the dynamic instability and therefore the dynamic behaviour of the dielectric properties of microtubule.

## REFERENCE

**[1]** Mandelkow, Eckhard and Eva-Maria Mandelkow, "Microtubule structure," Curr. Opin. In Struct. Biol., 171–179, 1994.

[2] Mershin, A., A. A. Kolomenski, H. A. Schuessler, and D. V. Nanopoulos. Tubulin dipole moment, dielectric constant and quantum behavior: computer simulations, experimental results and suggestions. Biosystems, 2004.

[3] Tuszynski, J.A., J.A. Brown, E. Crawford, E.J. Carpenter, M.L.A. Nip, J.M. Dixon, and M.V. Satariat, "Molecular dynamics simulations of tubulin structure and calculations of electrostatic properties of microtubules," Mathematical and Computer Modelling, 41, 1055 – 1070, 2005.

[4] Pokornŷ, J., jiří hašek and františek jelínek, "Electromagnetic Field of Microtubules: Effects on Transfer of Mass Particles and Electrons," Jour. of Biol. Phys. 31, 501 – 514, 2005.

[5] Havelka, D., M. Cifra, J. Vrba, "Cellular Radiofrequency Electromagnetic Field can be measured only by Nanotechnological Methods," ISMOT, Prague, 2011.

[6] Krivosudsky, Ondrej "Microwave absorption and permittivity of protein and microtubule solution," Master Thesis, Czech Technical University, Prague, 2014.

[7] Sahu, Satyajit, Subrata Ghosh, Daisuke Fujita, and Anirban Bandyopadhyay, "Live visualizations of single isolated tubulin protein self-assembly via tunneling current: effect of

electromagnetic pumping during spontaneous growth of microtubule," Scientific Reports, 4: 7303, 1–9, December 2014.

[8] Setayandeh, S. S., and A. Lohrasebi, "Influence of GHz electric fields on the mechanical properties of a microtubule," J Mol Model, 21, 1–7, March 2015.

[9] M. Cifra, D. Havelka, M. A. Deriu, "Electric field generated by longitudinal axial microtubule vibration modes with high spatial resolution microtubule model*,"* J. Phys. Conf. Ser., Vol. 329, 2011.

[10] Havelka, D., M. Cifra, O. kučera, "Multi-mode electro-mechanical vibrations of a microtubule: In silico demonstration of electric pulse moving along a microtubule," Appl. Phys. Lett. 104, 243702: 1-4, June 2014.

[11] Pokorný J., Jandová A., Nedbalová M., Jelínek F., Cifra M., Kučera O., Havelka D., Vrba J., Vrba J. Jr., Čoček A., Kobilková J., "Mitochondrial Metabolism – Neglected Link of Cancer Transformation and Treatment," Prague Medical Report, Vol. 113, No. 2, 81-84, 2012.

[12] Hameroff, S., and Roger Penrose, "Consciousness in the universe A review of the 'Orch OR'theory," Physics of Life Reviews 11, 39 – 78, March 2014.

[13] Vassilev, P.M., Dronzine, R.T., Vassileva, M.P., Georgiev, G.A., "Parallel arrays of microtubules in electric and magnetic fields," Biosci. Rep. 2, 1025–1029, 1982.

[14] Kirson, E.D., Gurvich, Z., Schneiderman, R., Dekel, E., Itzhaki, A., Wasserman, Y., Schatzberger, R., Palti, Y., "Disruption of cancer cell replication by alternating electric fields," Cancer Res. 64, 3288–3295, 2004.

[15] Minoura, I., Muto, E. "Dielectric measurement of individual microtubules using the electroorientation method," Biophys. J. 90, 3739–3748, 2006.

[16] Samsonov, A. and S. V. Popov "The Effect of a 94 GHz Electromagnetic Field on Neuronal Microtubules," Bioelectromagnetics 34, 133 – 144, 2013.

[17] Dye, R. B., "Taxol-induced Flexibility of Microtubules and Its Reversal by MAP-2 and Tau," The Jour. Of Biol. Chem., Vol. 268, No. 10, 6847 – 6850, 1993.

[18] Kovács, P. and G. Csaba "effect of drugs affecting microtubular assembly on microtubules, phospholipid synthesis and physiological indices (signalling, growth, motility and phagocytosis) in Tetrahymena pyriformis," Cell Biochem. Funct., Vol. 24, 419 – 429, May 2005.

[19] Zaremba, T. G., Thomas R. LeBon, David B. Millar, Ruthann M. Smejka1, and Robert J. Hawley, "Effects of Ultraviolet Light on the in Vitro Assembly of Microtubules," Biochemistry, Vol. 23, No. 6, 1073 – 1080, 1984.

[20] R., Veselská and Janisch R., "The Effect of UV Irradiation on Changes in Cytoskeleton and Viability of Mouse Fibroblasts L929 Cell Line," Scripta Medica (BRNO), Volume 73, No. 6, 393 – 408, Dec. 2000.

[21] Sahu, Satyajit, Subrata Ghosh, Kazuto Hirata, Daisuke Fujita, and Anirban Bandyopadhyay, "Multi-level memory-switching properties of a single brain microtubule," Appl. Phys. Lett., Vol. 102, 123701: 1–4, March 2013.

[22] Lahav, M., M. Abu-Abied, E. Belausov, A. Schwartz, and E. Sadot, "Microtubules of Guard Cells are Light Sensitive," Plant Cell Physiol., Vol. 45, No. 5, 573 – 582, 2004.

[23] Sambade,A., Amitesh Pratap, H. Buschmann, Richard J. Morris, and Clive Lloyd, "The Influence of Light on Microtubule Dynamics and Alignment in the Arabidopsis Hypocotyl," The Plant Cell, Vol. 24, No. 1, 192 – 201, January 2012.

[24] Sahu, Satyajit, S. Ghosh, B. Ghosh, K. Aswani, K. Hirata, D. Fujita, A. Bandyopadhyay, "Atomic water channel controlling remarkable properties of a single brain microtubule: Correlating single protein to its supramolecular assembly," Biosensors and Bioelectronics, Vol. 47, 141–148, March 2013.

[25] Kajfez, Darko, and Pierre Guillon, "Dielectric Resonators," Second Edition, Noble Publishing Corporation, Atlanta, 1998.